

### **IN THE SPECIFICATION**

Please amend the specification as follows.

On page 27, lines 15-30, please amend the paragraph as follows.

#### **Selection of YAC clones for FISH probe synthesis**

YAC clones were selected from the Whitehead/MIT map of the relevant chromosome in the cytogenetic intervals within which the breakpoints were adjudged to lie. YACs were obtained from the HGMP Resource Centre, Babraham Bioincubator, Babraham, Cambridge, UK [\[\[http://www.hgmp.mrc.ac.uk/\]\]](http://www.hgmp.mrc.ac.uk/). Clone DNA was prepared by standard methods and PCR amplified using primers designed against consensus sequence elements within the archetypal *Alu* repeat, Breen et al, 1992. This "Alu-PCR" gives a representative spread of non-repetitive sequence over the full length of the YAC and generates a better FISH probe than native YAC DNA. Alu-PCR was performed using the Expand Long Template PCR kit (Roche). Cycling conditions: 94°C - 45s, 55°C - 30s, 68°C - 8min: 35 cycles. 68°C - 10min final extension.

On page 28, lines 12-33, please amend the paragraph as follows.

#### **Resolution of breakpoint position**

BAC clones corresponding to positive YAC regions were arranged into contigs by consulting the Washington University FPC [\[\[http://www.genome.wustl.edu/gsc/human/Mapping/index.shtml\]\]](http://www.genome.wustl.edu/gsc/human/Mapping/index.shtml), UCSC GoldenPath Draft Human Genome Browser [\[\[http://genome.ucsc.edu/goldenPath/hgTracks.html\]\]](http://genome.ucsc.edu/goldenPath/hgTracks.html) and Ensembl [\[\[http://www.ensembl.org/\]\]](http://www.ensembl.org/) databases. BAC clones were supplied by BACPAC Resources, Oakland, California, USA [\[\[http://www.chori.org/bacpac/\]\]](http://www.chori.org/bacpac/). Clone selection was biased to gene-containing BACs. Once a breakpoint-spanning BAC was identified, the position of the breakpoint in relation to candidate gene exons was determined by FISH probes generated from chromosome-specific library cosmids (HGMP Resource centre) or precisely positioned, repeat element-free long-range PCR products (Expand long range PCR kit, Roche; see below for primer sequences). Cycling

conditions: 94°C - 45s, 52°C - 30s, 68°C - 11min: 35 cycles. 68°C - 15min final extension. Cosmids were isolated by probing the appropriate chromosome-specific library filters (HGMP-RC) with isotopically labelled exon-specific PCR products.

On page 29, lines 1-20, please amend the paragraph as follows.

**Example 1: Molecular characterisation of chromosomal disruption and identification of disrupted gene from patient 1**

FISH experiments on chromosome 3p13 had narrowed the location of the breakpoint to a region including the large gene *SEMCAP3* (approximately 250kb genomic extent). Two BAC clones were selected from the tiling diagram of BAC clones placed on the human genome map backbone (June 2002 release of the 'BAC End Pairs' track on the UCSC Genome Browser).[[; <http://genome.cse.ucsc.edu/index.html?org=Human>)).]] These were RPCI-11 606p16 and RPCI-11 94j25. By FISH, these BAC clones flanked the breakpoint (the former translocated to the derived chromosome 8 and the latter remained on the derived chromosome 3). The position of these two BAC clones indicated that the breakpoint lay within the large (200kb) intron between exons 3 and 4 of the *SEMCAP3* gene (see Fig.2). Thus, the inventors inferred from these results that the *SEMCAP3* gene was directly disrupted by the 3p13 translocation event and, as such, is a candidate gene for the psychiatric disorder exhibited by the patient.

On page 31, lines 18-24, please amend as follows.

**3'UTR primers**

Primer A: TGCCACGTGTTAGCAGAAAG SEQ ID NO. 66

Primer B: TGCCTTTAACCAGATGAGGC SEQ ID NO. 67

**SHGC-12093 primers**

Primer A: TCTTGTGGGTCACAATTAGGC SEQ ID NO. 68

Primer B: TAAAAAGGTGCAGTTTCTTCAGC'. SEQ ID NO. 69

On page 32, under "Microsatellites used in associated study," lines 27-37, please amend as follows.

Microsatellites used in associated study

D8S549

Primer A: AAATGAATCTCTGATTAGCCAAC SEQ ID NO. 70

Primer B: TGAGAGCCAACCTATTTCTACC SEQ ID NO. 71

N33 microsatellite

Primer A: AGGCTGAGTGCCAAAAAGTA SEQ ID NO. 72

Primer B: CTTTAAGCTTGCTATTTGAAGGC SEQ ID NO. 73

D8S1992

Primer A: TTCATCGTCTGAACCTGG SEQ ID NO. 74

On page 33, lines 1-9, please amend as follows.

Primer B: ACACATTTCCTCTATGTTGC[[]]] (SEQ ID NO. 75) were chosen and used to type 25 mother-father-schizophrenic proband trios and 64 schizophrenic cases and 64 normal controls. The haplotypes derived from the trio study were examined for frequency bias in the case and control samples. Certain haplotypes are currently over-represented in the schizophrenic case genotypes compared to controls. Appropriate individuals with the haplotypes are currently being screened for mutations.

On page 34, lines 16-32, please amend as follows.

**PCR primers**

Long-range PCR for FISH probe templates:

Int2-3 GRIK4a; CAGGAGGTCCTGTGAAGCTC[[],] SEQ ID NO. 76.

Int2-3 GRIK4b; ACAGGGAAAGAAGCAAAGCA[.,] SEQ ID NO. 77.

GRIK4 exon region-specific PCR: screening of chromosome 11  
cosmid libraries:

Ex1a/a' a; AAAGCTAAGCGCAGGTGTGT[.,] SEQ ID NO. 78.

Ex1a/a' b; TTTCTGGGAGGCAACCATAG[.,] SEQ ID NO. 79.

Ex1b a; GCAGAGTTATGTCATGCCCA[.,] SEQ ID NO. 80.

Ex1b b; CCTGTGCAGCACTCTGATGT[.,] SEQ ID NO. 81.

Ex2/3 a; TTGAACCAAGAGAACAGGG[.,] SEQ ID NO. 82.

Ex2/3 b; TCCCCTTCTCCTTCCAGTTT SEQ ID NO. 83.

Cycling conditions: 94°C – 2min initial denaturation. 94°C – 1min, 52°C – 1min, 72°C – 75s: 33  
cycles. 72°C – 15min final extension.

On page 38, lines 7-22, please amend the paragraph as follows.

#### **Fine FISH mapping of the breakpoint with cosmid clones**

PCR products corresponding to regions in or near *hNPAS3* exons 4, 5 and 6 were obtained using the following primers under standard PCR conditions (Exon 4-*i* ACAACCATTTCTGGGAACAGC (SEQ ID NO. 84), Exon 4-*ii* GTGTAGGGAAAGCCATCCAA (SEQ ID NO. 85), Exon 5-*i* TCTTTTTCCTGCAGTCCCTG (SEQ ID NO. 86), Exon 5-*ii* CTCCAAATGACTCCTGCCAT (SEQ ID NO. 87), Exon 6-*i* GCCTCTGCCATAGATTTTGC (SEQ ID NO. 88), Exon 6-*ii* TTCCTTCCCACCCTTTCTCT (SEQ ID NO. 89)). Probes were created by random-primed labelling of PCR products with radioactive dCTP; these were used to screen a LANL chromosome 14-specific cosmid library (LA14NC01 obtained from the UK HGMP Resource Centre, Hinxton, Cambridge) using [[hybridizing]]hybridizing conditions set out in Church and Gilbert (1986). Positive clones (exon; LA1431-G5, exon 5: LA14123 - C4 and exon 6; LA1487 - D9) were prepared by a standard alkaline lysis protocol and taken through FISH analysis as above.

On page 39, lines 31-33, please amend as follows.

Long-range PCR primers – NPAS3 exon 5

- a) ccagcttgatgtggtgtgg SEQ ID NO. 90
- b) ttactcccagtgcccattgt[.] SEQ ID NO. 91.

On page 43, lines 4-10, please amend the paragraph as follows.

#### **PCR primers**

Long-range PCR for FISH probe templates:

PDE4B3a; GTCAGACAAATCCAAATGGAGAG (SEQ ID NO. 92), PDE4B3b;  
CTTTCTCCTGTCACTTTCCTTCA (SEQ ID NO. 93).

Cycling conditions: 94°C - 2min initial denaturation. 94°C - 1min, 52°C - 1min, 72°C - 75s: 33 cycles.  
72°C - 15min final extension.

On page 45, line 23 through page 47, line 14, please amend the paragraph as follows.

#### **Example 6: Molecular characterisation of chromosomal disruption and identification of disrupted gene from patient 4**

FISH experiments on chromosome 16q21 had narrowed the location of the breakpoint to a region including the large gene *CDH8* (approximately 400kb genomic extent). Three BAC clones were selected from the tiling diagram of BAC clones placed on the human genome map backbone (June 2002 release of the 'BAC End Pairs' track on the UCSC Genome Browser).[;  
<http://genome.cse.ucsc.edu/index.html?org=Human>.)] These were RPCI-11 599c11, RPCI-11 875e12 and RPCI-11 685m21. By FISH, these BAC clones flanked the breakpoint (the first two translocated the derived chromosome 1 whereas the third remained on the derived chromosome 16). The position of these three BAC clones indicated that the breakpoint lay within the large (100kb) intron between exons 1 and 2 of the *CDH8* gene (see Fig.2). Thus, the inventors inferred from these results that the *CDH8* gene was directly disrupted by the 16q21 translocation event and, as such, is a candidate gene for the psychiatric disorder exhibited by the patient. The similar disruption of the

*PDE4B* gene on chromosome 1 and their relative orientations on the two chromosomes raised the possibility that the derived chromosomes (the two chromosomes resulting from the translocation: der(1) and der(16)) could transcribe fusion/hybrid genes. This has been frequently seen in cases where a translocation gives rise to susceptibility to cancers. In essence, the translocation in the proband resulted in an exchange of the two genes= promoter and first exon sequences. On the der(1) the promoter and first exon of the *CDH8* gene are juxtaposed to exon 2 and downstream of the *PDE4B* gene (see Fig.33). However, the reading frames of these two gene segments are not the same, resulting in a prematurely truncated peptide with only the signal peptide, proprotein fragment and a small portion of the cadherin domain contained within (see Fig. 37a). This would be expressed in the same cell types/tissues as the normal *CDH8* gene but the functional/pathological significance of this small peptide is not clear at the current time. On the der(16) the *PDE4B* promoter and exon 1a are juxtaposed to exon 2 and downstream of the *CDH8* gene (see Fig.33). Exon 1a of *PDE4B* does not contain a translation start-site so the reading frame compatibilities of the putative fused transcript are not an issue. However, exon 2 and downstream of the *CDH8* gene contain several ATG start-sites which could be employed by translational machinery to generate peptide sequences. In two of the reading frames, any generated peptides would be small and probably of no consequence. The third reading frame (the normal *CDH8* reading frame, see Fig.5b) contains three ATG start-sites early on, with the second of these forming a very good match to the canonical Kozak sequence found at most translation start-sites (CCAxx**ATGG**) (SEQ ID NO. 94). If this one is used then the resulting peptide will be identical to normal CDH8 protein but lacking the N-terminal portion encoding the signal peptide, proprotein fragment, the first cadherin domain and most of the second cadherin domain. Although the bulk of the peptide sequence is as the normal CDH8 protein, the lack of the N-terminal sequences may prevent the protein from entering the Golgi/ER subcellular compartments - a process that is required for the correct insertion in/trafficking to the cell membrane. The functional/pathological consequence of the presence of this truncated form of the CDH8 protein in the cytoplasm of tissues where the long form of the *PDE4B* gene is expressed is uncertain at this point.